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## SPECIFICATION

N-HYDROXYFORMAMIDINE DERIVATIVES

## 5 TECHNICAL FIELD

The present invention relates to N-(4-alkyl-substituted phenyl)-N'-hydroxyformamidine derivatives which inhibit enzymes mediating the production of 20-hydroxyeicosatetraenoic acid (20-HETE) biosynthesized from  
10 arachidonic acid.

## BACKGROUND ART

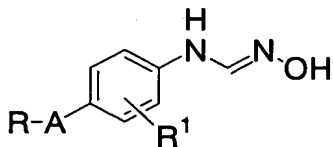
Prostaglandins produced by cyclooxygenase and leukotrienes produced by lipoxygenase are widely known as  
15 physiologically active substances produced from arachidonic acid. However, recent studies have been clarifying a wide variety of *in vivo* functions of 20-HETE which is produced from arachidonic acid by the action of enzymes belonging to the cytochrome p450 family. Previous reports have  
20 indicated that 20-HETE causes microvascular constriction or dilation in major organs (e.g., kidneys, cerebral blood vessels) and induces cell proliferation, thus suggesting that 20-HETE plays an important physiological role *in vivo* and is deeply involved in the development of various  
25 conditions such as kidney diseases, cerebrovascular diseases and cardiovascular diseases (J. Vascular Research, vol. 32, p. 79, 1995, Am. J. Physiol., vol. 277, p. R607, 1999, Physiol. Rev., vol. 82, p. 131, 2002).

Under these circumstances, it has been reported that some N'-hydroxyphenylformamidine derivatives and carboxylic acid derivatives have a strong inhibitory effect against 20-HETE-producing enzymes (International Patent Publication Nos. WO0132164, WO01096309 and WO0168610, Japanese Patent Publication Nos. JP01354658 and JP01354646).

#### DISCLOSURE OF THE INVENTION

The object of the present invention is to provide an agent for inhibiting the production of 20-HETE, which has been shown to be involved in the development of microvascular constriction or dilation in major organs (e.g., kidneys, cerebral blood vessels) or in inducing cell proliferation, etc.

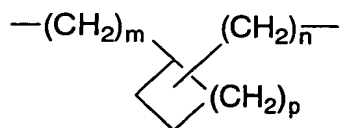
As a result of extensive and intensive efforts made to achieve the object stated above, the inventors of the present invention have found that aromatic compounds having a specific partial structure, i.e., N-(4-alkyl-substituted phenyl)-N'-hydroxyformamidine derivatives unexpectedly have a selective inhibitory effect against 20-HETE-producing enzymes. This finding led to the completion of the present invention. Namely, the present invention provides an N-hydroxyformamidine derivative of the following formula or a pharmaceutically acceptable salt thereof:



(wherein

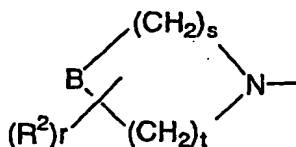
$R^1$  represents a hydrogen atom, a  $C_{1-4}$  alkyl group, a  $C_{1-4}$  alkoxy group or a halogen atom,

A represents a  $C_{1-10}$  alkylene group or a group of the following formula:



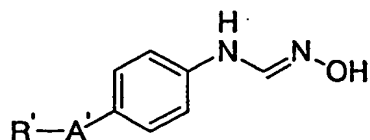
(wherein m, n and p each represent an integer of 0 to 4), and

10 R represents an N,N-di- $C_{1-6}$  alkylamino group, a dioxanyl group, a  $C_{1-4}$  alkyl-substituted dioxanyl group, a  $C_{1-4}$  alkoxy- $C_{1-4}$  alkoxy group or a group of the following formula:



15 (wherein s and t each represent an integer of 1 to 4, B represents a methylene group, an oxygen atom, a sulfur atom, a nitrogen atom, a  $C_{1-4}$  alkyl-substituted nitrogen atom, a phenyl-substituted nitrogen atom or a benzyl-substituted nitrogen atom,  $R^2$  represents a hydrogen atom or a  $C_{1-4}$  alkyl group, and r represents an integer of 0 to 2)).

In another aspect, the present invention provides an N-hydroxyformamidine derivative of the following formula or a pharmaceutically acceptable salt thereof:



(wherein A' represents a C<sub>1-10</sub> alkylene group and R' represents an N,N-di-C<sub>1-6</sub> alkylamino group, a pyrrolidinyl group, a dioxanyl group, a C<sub>1-4</sub> alkyl-substituted dioxanyl group or a C<sub>1-4</sub> alkoxy-C<sub>1-4</sub> alkoxy group).

In another aspect, the present invention provides a pharmaceutical preparation which comprises an N-hydroxyformamidinium derivative or a pharmaceutically acceptable salt thereof as an active ingredient.

In another aspect, the present invention provides a N-hydroxyformamidinium derivative which is an inhibitor of a 20-HETE-producing enzyme.

In another aspect, the present invention provides a therapeutic agent for kidney diseases, cerebrovascular diseases or cardiovascular diseases, which comprises an N-hydroxyformamidinium derivative or a pharmaceutically acceptable salt thereof as an active ingredient.

The terms used herein are defined as follows.

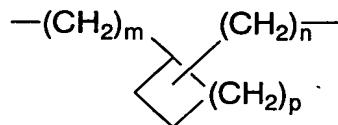
As used herein, the designation "C<sub>x-y</sub>" is intended to mean a group having x to y carbon atoms.

The term "C<sub>1-4</sub> alkyl group" is intended to mean a linear or branched alkyl group having 1 to 4 carbon atoms. Examples include a methyl group, an ethyl group, a n-propyl group, an isopropyl group and a n-butyl group. The term "C<sub>1-4</sub> alkoxy group" is intended to mean a linear or branched

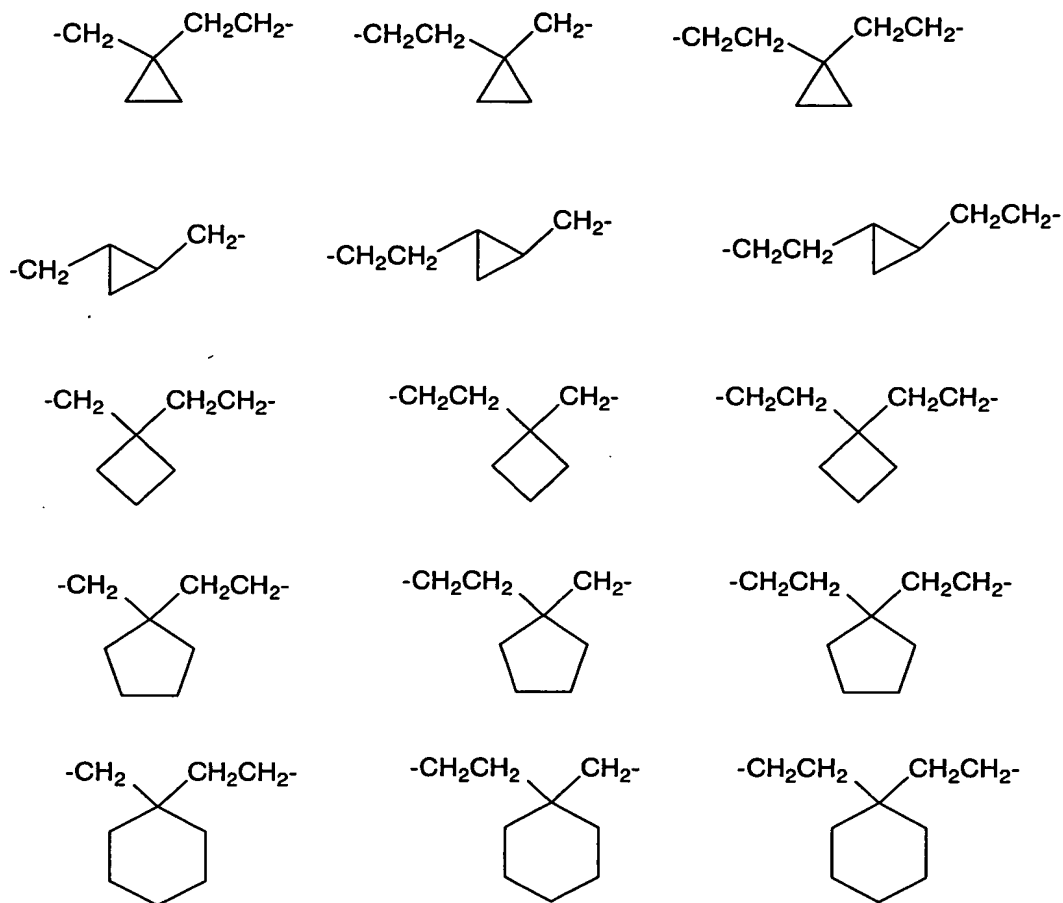
alkoxy group having 1 to 4 carbon atoms. Examples include a methoxy group, an ethoxy group, a n-propoxy group, an isopropoxy group and a n-butoxy group.

The term "N,N-di-C<sub>1-6</sub> alkylamino group" is intended to include an N,N-dimethylamino group, an N,N-diethylamino group and the like.

A group of the following formula:



10 refers to a C<sub>3-7</sub> cycloalkane modified to have two alkylene groups or binding hands. Preferred are those in which p is 0 to 3, and m and n are each 1 to 3, as exemplified by the groups shown below.



The term "dioxanyl group" is intended to mean a saturated 6-membered ring having two oxygen atoms as heteroatoms (dioxane), preferably a monovalent group derived by removing hydrogen from the 1,3-dioxane ring.

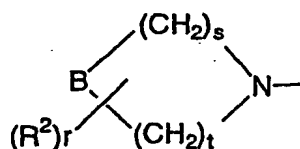
With respect to the term "C<sub>1-4</sub> alkyl-substituted dioxanyl group," its ring may be substituted with a C<sub>1-6</sub> alkyl group. Examples include a 5,5-dimethyl-1,3-dioxan-2-yl group.

The term "C<sub>1-4</sub> alkoxy-C<sub>1-4</sub> alkoxy group" is intended to mean a structure composed of two C<sub>1-4</sub> alkoxy groups. Preferred examples include a methoxyethoxy group, an

ethoxyethoxy group, a n-butoxyethoxy group and an isobutoxyethoxy group.

The term "C<sub>1-10</sub> alkylene group" is intended to mean a linear or branched alkylene group having 1 to 10 carbon atoms. Examples include a methylene group, a methylenemethylene group, an ethylene group, a propylene group, a heptylene group, a 2,2-dimethylpropylene group, a 3,3-dimethylpropylene group and a hexylene group. Among them, preferred are an ethylene group and a 3,3-dimethylpropylene group.

A group of the following formula:



is intended to include a pyrrolidino group, a piperidino group, a morpholino group, a thiomorpholino group, a 4-methylpiperazin-1-yl group and a 3,5-dimethylmorpholino group.

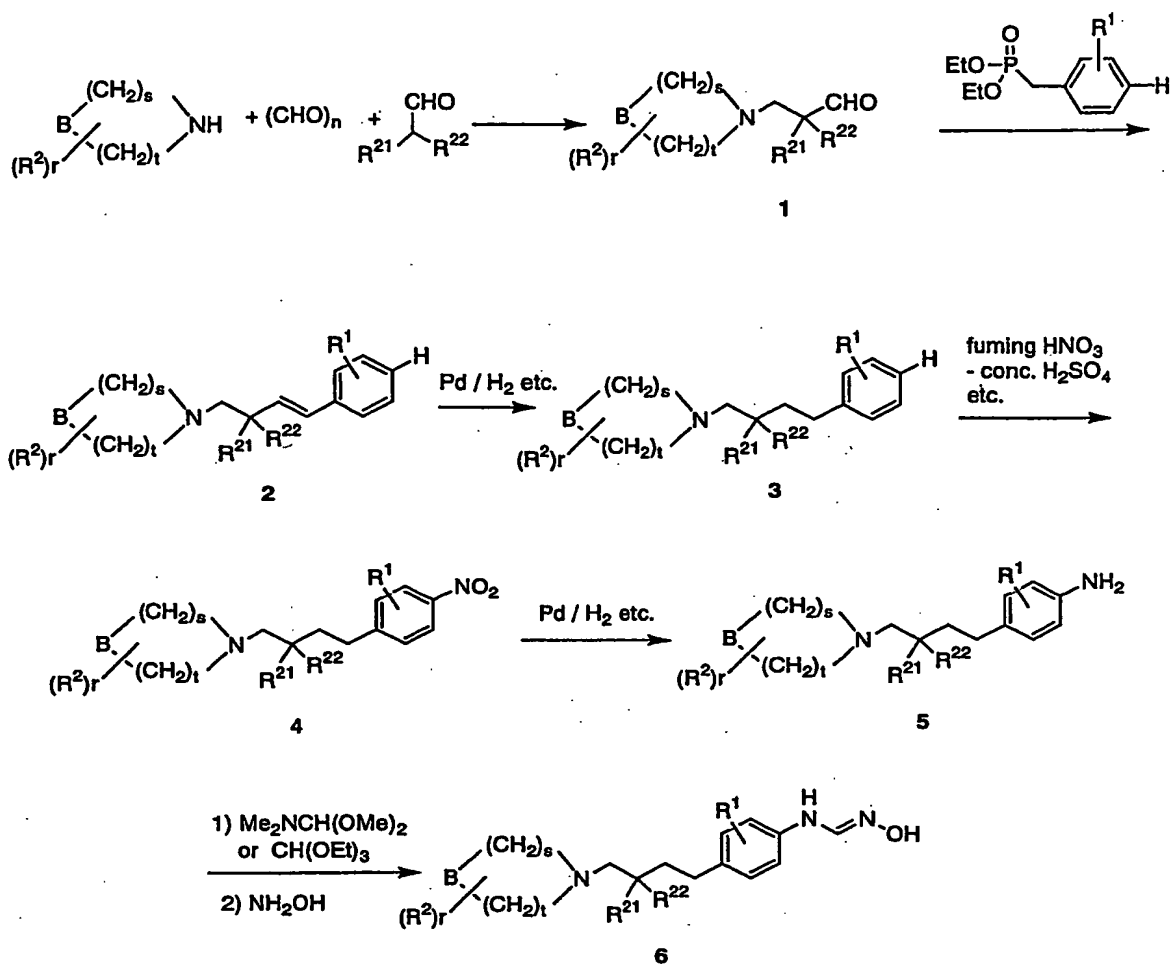
In addition, the term "pharmaceutically acceptable salt" is intended to mean, for example, a salt with an alkali metal, an alkaline earth metal, ammonium or an alkylammonium, or a salt with a mineral acid or an organic acid. Examples include a sodium salt, a potassium salt, a calcium salt, an ammonium salt, an aluminum salt, a triethylammonium salt, an acetate, a propionate, a butyrate, a formate, a trifluoroacetate, a maleate, a tartrate, a citrate, a stearate, a succinate, an ethylsuccinate, a

lactobionate, a gluconate, a glucoheptate, a benzoate, a  
methanesulfonate, an ethanesulfonate, a 2-  
hydroxyethanesulfonate, a benzenesulfonate, a p-  
toluenesulfonate, a lauryl sulfate, a malate, an aspartate,  
5 a glutamate, an adipate, a salt with cysteine, a salt with  
N-acetylcysteine, a hydrochloride, a hydrobromide, a  
phosphate, a sulfate, a hydroiodide, a nicotinate, an  
oxalate, a picrate, a thiocyanate, an undecanoate, a salt  
with an acrylate polymer and a salt with a carboxyvinyl  
10 polymer.

The compound of the present invention can be  
synthesized in the following manner, by way of example.

Production Process 1





(wherein B,  $R^1$ ,  $R^2$ , r, s and t are as defined above,  $R^{21}$  and  $R^{22}$  each represent a  $C_{1-4}$  alkyl group or together form a  $C_{3-7}$  cycloalkane ring).

- 5            Aldehyde 1 synthesized via Mannich reaction and the Horner-Emmons reagent (e.g., diethyl benzylphosphonate) may be reacted at  $-78^\circ C$  to room temperature for 1 to 24 hours in an appropriate solvent (e.g., tetrahydrofuran, ether, toluene, N,N-dimethylformamide) and in the presence of an
- 10           appropriate base (e.g., n-butyllithium, triethylamine, N,N-diisopropylethylamine, sodium hydride, sodium methoxide, potassium tert-butoxide) to prepare Compound 2. Compound 2

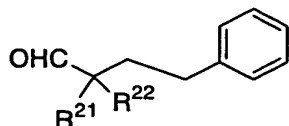
may then be reacted under a hydrogen atmosphere in the presence of a catalyst (e.g., palladium-activated charcoal, palladium hydroxide) and in an appropriate solvent (e.g., methanol, ethanol, propanol, tetrahydrofuran, dioxane, toluene, methylene chloride, chloroform, acetonitrile, ethyl acetate) to prepare Compound 3. Compound 3 may then be nitrated under certain conditions, e.g., fuming nitric acid-concentrated sulfuric acid, potassium nitrate-concentrated sulfuric acid, or cerium(IV) diammonium nitrate-concentrated sulfuric acid, to prepare a nitro derivative (4). Compound 4 may then be treated in an appropriate solvent (e.g., methanol, ethanol, propanol, tetrahydrofuran, dioxane, toluene, methylene chloride, chloroform, acetonitrile, ethyl acetate) using a reducing agent (e.g., palladium-activated charcoal/under a hydrogen atmosphere, palladium-activated charcoal/hydrazine hydrate, palladium-activated charcoal/ammonium formate, tin(II) chloride monohydrate, iron/ammonium chloride, Raney Nickel/hydrazine hydrate, preferably palladium-activated charcoal/under a hydrogen atmosphere) to reduce the nitro group, thereby preparing an aniline derivative (5).

Subsequently, Compound 5 may be reacted with N,N-dimethylformamide dimethyl acetal at room temperature to 150°C, preferably 70°C to 100°C, for 2 to 72 hours in an appropriate solvent (e.g., methanol, ethanol, propanol, tetrahydrofuran, dioxane, toluene, methylene chloride, chloroform, acetonitrile, ethyl acetate). The resulting intermediate may be treated with hydroxylammonium chloride

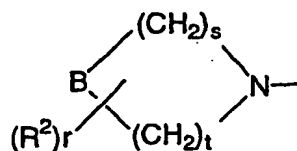
in an appropriate solvent (e.g., methanol, ethanol, propanol, tetrahydrofuran, dioxane, toluene, methylene chloride, chloroform, acetonitrile, ethyl acetate) to prepare Compound 6 according to the present invention.

5 Alternatively, Compound 5 may also be reacted with an orthoformate ester (e.g., trimethyl orthoformate, triethyl orthoformate) in the presence or absence of a catalytic amount of an organic acid (e.g., acetic acid), a mineral acid (e.g., hydrochloric acid), pyridine hydrochloride or  
10 the like to give an intermediate. The reaction temperature ranges from room temperature to 150°C, preferably 70°C to 100°C, while the reaction time ranges from 2 to 72 hours. Either with or without isolation, the intermediate may be treated with hydroxylamine in an appropriate solvent (e.g.,  
15 methanol, ethanol, propanol, tetrahydrofuran, dioxane, toluene, methylene chloride, chloroform, acetonitrile, ethyl acetate) to prepare Compound 6 according to the present invention.

Alternatively, a compound of the following formula  
20 (synthesized as described in J Org Chem vol. 53, p. 3309, 1988):



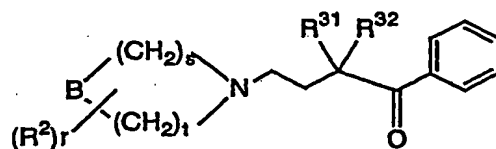
(wherein R<sup>21</sup> and R<sup>22</sup> are as defined above) and an amine of the following formula:



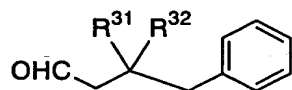
(wherein B,  $R^2$ , r, s and t are as defined above) may be subjected to reductive alkylation to give Synthetic Intermediate 3. Using this synthetic intermediate, the same procedure may be repeated to prepare Compound 6 according to the present invention.

#### Production Process 2

A compound of the following formula (synthesized as described in Org Letter vol. 4, p. 1951, 2002):

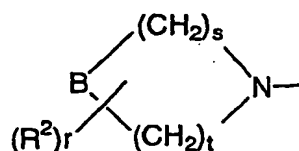


(wherein B,  $R^2$ , r, s and t are as defined above, and  $R^{31}$  and  $R^{32}$  each represent a  $C_{1-4}$  alkyl group or together form a  $C_{3-7}$  cycloalkane ring) may be reduced via Wolff-Kishner reaction or the like, or alternatively, a compound of the following formula (synthesized as described in Tetrahedron, vol. 46, p. 2111, 1990):

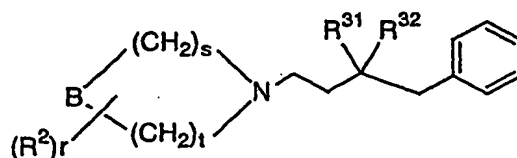


(wherein  $R^{31}$  and  $R^{32}$  are as defined above) and an amine of

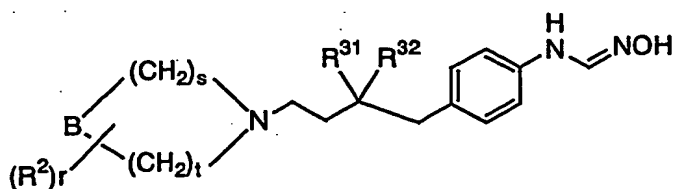
the following formula:



(wherein B, R<sup>2</sup>, r, s and t are as defined above) may be subjected to reductive alkylation to give a synthetic  
5 intermediate of the following formula:

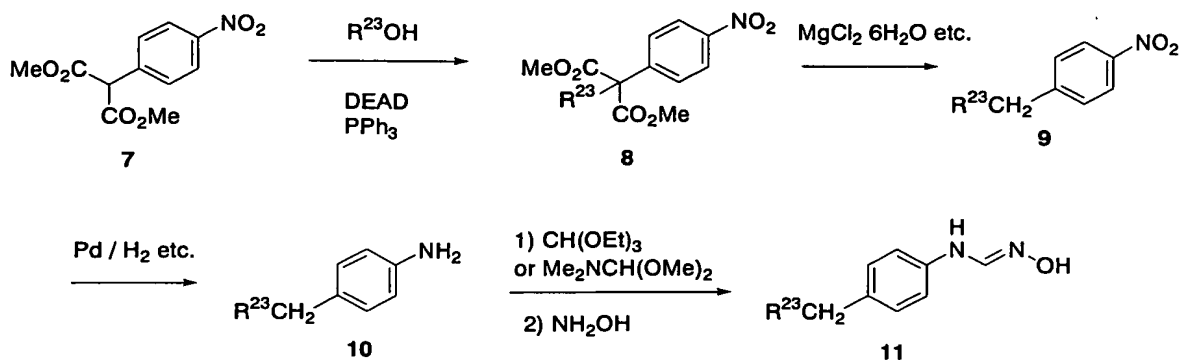


(wherein B, R<sup>2</sup>, r, s, t, R<sup>31</sup> and R<sup>32</sup> are as defined above).  
Using this synthetic intermediate, the same procedure as shown in Production Process 1 may be repeated to synthesize  
10 the compound of the present invention represented by the following formula:

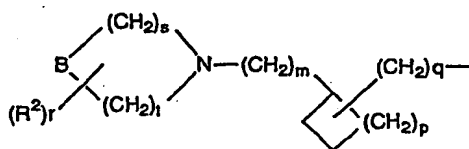


(wherein B, R<sup>2</sup>, r, s, t, R<sup>31</sup> and R<sup>32</sup> are as defined above).

15 Production Process 3



2-(4-Nitrophenyl)malonic acid dimethyl ester (7) may be condensed with an alcohol of the formula  $R^{23}OH$  (wherein  $R^{23}$  represents a  $C_{1-4}$  alkoxy- $C_{1-3}$  alkyl group, an N,N-di- $C_{1-6}$  alkylamino- $C_{1-9}$  alkyl group or a group of the following formula:



(wherein B,  $R^2$ , r, s, t, m and p are as defined above, and q represents an integer of 0 to 3)) via Mitsunobu reaction to synthesize Compound 8 (Tetrahedron Lett., vol. 42, p. 8395, 2001). Compound 8 may then be treated with NaCl or  $MgCl_2 \cdot 6H_2O$  in an appropriate solvent (e.g., N,N-dimethylacetamide, DMSO, DMSO- $H_2O$ ) to synthesize Compound 9. The reaction temperature ranges from  $100^\circ C$  to  $150^\circ C$ , and the reaction time ranges from 5 to 24 hours (Synthesis, vol. 12, p. 1659, 2000). Subsequently, the nitro group may be reduced in the same manner as shown in Production Process 1 to synthesize an aniline derivative (10). At the end of the process, hydroxyformamidine may be constructed in the same manner as shown in Production Process 1 to prepare

Compound 11 according to the present invention.

#### BEST MODE FOR CARRYING OUT THE INVENTION

The present invention will be further described in  
5 more detail in the following examples.

##### Example 1

Preparation of N-[4-(4-N,N-dimethylamino-3,3-dimethyl-  
butyl)phenyl]-N'-hydroxyformamidine

(1) To a mixture of dimethylamine hydrochloride (10.8 g,  
10 0.132 mol) and 2-propanol (62 ml), paraformaldehyde (8.7 g)  
and isobutyl aldehyde (12 ml, 0.132 mol) were added. The  
reaction mixture was stirred under heating at reflux  
overnight. After cooling to room temperature, the reaction  
mixture was concentrated. The residue was diluted with  
15 saturated aqueous sodium bicarbonate and extracted with  
ethyl acetate (100 ml  $\times$  5). The combined organic layers  
were dried over anhydrous magnesium sulfate and  
concentrated under reduced pressure to give 3-  
dimethylamino-2,2-dimethylpropionaldehyde (8.4 g) as a  
20 colorless oil.

(2) A mixture of diethyl benzylphosphonate (7.4 g, 0.0325  
mol) and THF (32 ml) was cooled to  $-78^{\circ}\text{C}$ , followed by  
addition of n-BuLi (1.6 M in n-hexane, 19.4 mL, 0.0311 mol)  
under a nitrogen stream. After 15 minutes, a solution of  
25 3-dimethylamino-2,2-dimethylpropionaldehyde (3.5 g, 0.0271  
mol) in THF (20 ml) was added dropwise. After stirring for  
30 minutes, the reaction mixture was warmed to room  
temperature, diluted with water (50 ml), and extracted

twice with ethyl acetate. The combined organic layers were washed with saturated aqueous sodium chloride, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by NH silica gel column chromatography (hexane:ethyl acetate = 96:4) to give (2,2-dimethyl-4-phenyl-3-butenyl)dimethylamine (3.3 g) as a colorless oil.

(3) A methanol solution of (2,2-dimethyl-4-phenyl-3-butenyl)dimethylamine (3.0 g, 0.015 mol) was stirred overnight in the presence of 10% palladium-activated charcoal (0.3 g) under a hydrogen atmosphere. The insoluble materials were removed by filtration through celite and the resulting filtrate was concentrated to give (2,2-dimethyl-4-phenylbutyl)dimethylamine (2.9 g) as a colorless crystal.

(4) To a mixture of (2,2-dimethyl-4-phenylbutyl)dimethylamine (2.9 g, 0.014 mol), concentrated sulfuric acid (15 ml) and chloroform (29 ml), fuming nitric acid (0.58 ml, 0.014 mol) was slowly added dropwise while vigorously stirring the mixture under ice cooling. After 10 minutes, 5M sodium hydroxide (90 ml) was added to adjust the reaction mixture to pH 8.0. The reaction mixture was further diluted with water (500 ml) and extracted twice with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified by NH silica gel column chromatography (hexane:ethyl acetate = 92:8) to give a mixture of [2,2-dimethyl-4-(4-



nitrophenyl)butyl]dimethylamine and [2,2-dimethyl-4-(2-nitrophenyl)butyl]dimethylamine (10:1; 0.45 g) as a yellow oil.

(5) A methanol solution of [2,2-dimethyl-4-(4-nitrophenyl)butyl]dimethylamine (400 mg, 1.60 mmol) was stirred in the presence of 10% palladium-activated charcoal (40 mg) under a hydrogen atmosphere for 3 hours. The insoluble materials were removed by filtration through celite and the resulting filtrate was concentrated to give 4-(4-dimethylamino-3,3-dimethylbutyl)aniline (290 mg) as a red oil.

Subsequently, to a solution of 4-(4-dimethylamino-3,3-dimethylbutyl)aniline (200 mg, 0.908 mmol) in methanol (4 ml), N,N-dimethylformamide dimethyl acetal (0.48 ml, 3.6 mmol) was added and then stirred under heating at reflux overnight. After cooling to room temperature, the reaction mixture was mixed with hydroxylammonium chloride (315 mg, 4.54 mmol) and stirred at room temperature for 78 hours. The reaction mixture was concentrated, followed by the addition of 2.5 M sodium hydroxide, and then, extracted three times with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was recrystallized from a mixed solvent of hexane and ethyl acetate to give the titled compound (68 mg) as a colorless powder.

<sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>) δ:

0.87 (s, 6H), 1.39-1.45 (m, 2H), 2.08 (s, 2H), 2.23 (s, 6H),

2.38-2.44 (m, 2H), 6.98-7.05 (m, 4H), 7.38 (d,  $J = 10.7$  Hz, 1H), 8.39 (d,  $J = 10.7$  Hz, 1H), 9.73 (s, 1H)

Melting point: 124.0-126.0°C

5 Example 2

Preparation of N-[4-(4-N,N-diethylamino-3,3-dimethyl-butyl)phenyl]-N'-hydroxyformamidine

The same procedure as used in Example 1 was repeated to synthesize the titled compound.

10  $^1\text{H}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$ :

0.86 (s, 6H), 0.93 (t,  $J = 7.2$  Hz, 6H), 1.42-1.36 (m, 2H), 2.17 (s, 2H), 2.39-2.45 (m, 2H), 2.47-3.31 (m, 4H), 6.98-7.05 (m, 4H), 7.38 (d,  $J = 10.7$  Hz, 1H), 8.39 (d,  $J = 10.7$  Hz, 1H), 9.75 (s, 1H)

15 Melting point: 95.5-97.5°C (colorless powder)

Example 3

Preparation of N-[4-(4-pyrrolidin-1-yl-3,3-dimethyl-butyl)phenyl]-N'-hydroxyformamidine

20 The same procedure as used in Example 1 was repeated to synthesize the titled compound.

$^1\text{H}$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$ :

1.40-1.46 (m, 2H), 1.64-1.68 (m, 4H), 2.29 (s, 2H), 2.53-2.58 (m, 2H), 6.98-7.05 (m, 4H), 7.38 (d,  $J = 10.7$  Hz, 1H), 8.39 (d,  $J = 10.7$  Hz, 1H), 9.75 (s, 1H)

25 Melting point: 120.0-121.5°C (colorless powder)

Example 4

Preparation of N-[4-(4-N,N-diethylaminobutyl)phenyl]-N'-hydroxyformamidine

(1) To a solution of 4-(4-nitrophenyl)butyric acid (1.0 g, 4.78 mmol) and diethylamine (0.699 g, 9.56 mmol) in N,N-dimethylformamide (10 ml), N-ethyl-N'-3-dimethylaminopropylcarbodiimide hydrochloride (1.37 g, 7.17 mmol) and 1-hydroxybenzotriazole monohydrate (0.969 g, 7.17 mmol) were added and stirred at room temperature for 24 hours. After addition of water, the reaction mixture was extracted twice with ethyl acetate. The combined organic layers were washed with saturated aqueous sodium bicarbonate, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure to give N,N-diethyl-4-(4-nitrophenyl)butylamide (1.4 g) as a light-yellow oil.

To a mixture of N,N-diethyl-4-(4-nitrophenyl)butylamide (1.4 g) and THF (14 ml), 1M  $\text{BH}_3 \cdot \text{THF}$  (9.6 ml) was added dropwise and stirred overnight at room temperature. After 1M  $\text{BH}_3 \cdot \text{THF}$  (7.0 ml) was further added dropwise, the reaction mixture was stirred at room temperature for 6 hours, followed by addition of 50% acetic acid (3 ml). The reaction mixture was concentrated, followed by the addition of saturated aqueous sodium bicarbonate, and then, extracted twice with ethyl acetate. The combined organic layers were washed with saturated aqueous sodium chloride, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure to give diethyl-[4-(4-nitrophenyl)butyl]amine (1.74 g) as a

light-yellow oil.

(3) A methanol solution of diethyl-[4-(4-nitrophenyl)butyl]amine (1.74 g) was stirred in the presence of 10% palladium-activated charcoal (0.3 g) under a hydrogen atmosphere for 3 hours. The insoluble materials were removed by filtration through celite and the resulting filtrate was concentrated. The residue was purified by NH silica gel column chromatography (hexane:ethyl acetate = 2:1) to give 4-(4-diethylaminobutyl)-aniline (998 mg) as an oil.

(4) To a solution of 4-(4-diethylaminobutyl)-aniline (676 mg, 3.12 mmol) in methanol (4 ml), N,N-dimethylformamide dimethyl acetal (0.83 ml, 6.2 mmol) was added. The reaction mixture was stirred under heating at reflux for 1 hour. After cooling to room temperature, the reaction mixture was mixed with hydroxylammonium chloride (433 mg, 6.24 mmol) and stirred at room temperature for 90 hours. The reaction mixture was concentrated, diluted with saturated aqueous sodium bicarbonate, and extracted twice with ethyl acetate and five times with chloroform. The combined organic layers were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified by NH silica gel column chromatography (hexane:ethyl acetate = 5:95). The resulting solid was recrystallized from a mixed solvent of hexane and ethyl acetate to give the titled compound (314 mg) as a colorless powder.

$^1\text{H-NMR}$  (300 MHz,  $\text{DMSO-d}_6$ )  $\delta$ :

0.92 (t,  $J = 7.3$  Hz, 6H), 1.24-1.60 (m, 4H), 2.26-2.45 (m, 8H), 6.99-7.08 (m, 4H), 7.39 (d,  $J = 11.0$  Hz, 1H), 8.42 (d,  $J = 11.0$  Hz, 1H), 9.77 (s, 1H)

Melting point: 110.0-113.0°C

5

#### Example 5

Preparation of N-{4-[3-(2-methoxyethoxy)propyl]phenyl}-N'-hydroxyformamidine

(1) To a solution of triphenylphosphine (4.81 g, 18.3 mmol) in tetrahydrofuran (20 ml) cooled to 0°C, diethyl azodicarboxylate (40% in toluene; 7.99 g, 18.3 mmol) and diethylene glycol monomethyl ether (2.21 g, 18.4 mmol) were added sequentially. The reaction mixture was warmed to room temperature and stirred for 30 minutes. After cooling to 0°C again, a solution of 2-(4-nitrophenyl)malonic acid dimethyl ester (3.10 g, 12.2 mmol) in tetrahydrofuran (50 ml) was added dropwise. The reaction mixture was warmed to room temperature, stirred overnight at room temperature, and then reacted at 60°C for 5.5 hours. After addition of ethyl acetate, the reaction mixture was washed with saturated aqueous sodium chloride, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane:ethyl acetate = 4:1 to 2:1), followed by NH silica gel column chromatography (hexane:ethyl acetate = 4:1) to give 2-[2-(2-methoxyethoxy)ethyl]-2-(4-nitrophenyl)malonic acid dimethyl ester (1.80 g) as a light-yellow oil.

(2) To a solution of 2-[2-(2-methoxyethoxy)ethyl]-2-(4-nitrophenyl)malonic acid dimethyl ester (1.13 g, 3.2 mmol) in N,N-dimethylacetamide (6 ml), magnesium chloride hexahydrate (1.30 g, 6.4 mmol) was added and reacted at 5 150°C for 10 hours. The reaction mixture was cooled to room temperature, diluted with ethyl acetate, and washed with saturated aqueous sodium chloride. The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was purified 10 by silica gel column chromatography (hexane:ethyl acetate = 4:1 to 2:1) to give 1-[3-(2-methoxyethoxy)propyl]-4-nitrobenzene (0.52 g) as a yellow oil.

(3) A solution of 1-[3-(2-methoxyethoxy)propyl]-4-nitrobenzene (0.49 g, 2.1 mmol) in methanol (10 ml) was 15 stirred in the presence of 5% palladium-activated charcoal (0.20 g) under a hydrogen atmosphere at room temperature for 1 hour. After confirming the disappearance of starting materials by TLC analysis, the insoluble materials were removed by filtration using celite and the filtrate was 20 concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane:ethyl acetate = 7:3 to 1:1) to give 4-[3-(2-methoxyethoxy)propyl]phenylamine (0.38 g) as a light-yellow oil.

25 (4) To a solution of 4-[3-(2-methoxyethoxy)propyl]phenylamine (0.20 g, 0.97 mmol) in methanol (5 ml), N,N-dimethylformamide dimethyl acetal (0.24 g, 2.0 mmol) was added and reacted at 100°C for 4.5

hours. After cooling to room temperature, the reaction mixture was mixed with hydroxylamine monohydrochloride (0.14 g, 1.9 mmol) and stirred overnight at room temperature. After further addition of hydroxylamine monohydrochloride (0.067 g, 0.96 mmol), the reaction mixture was reacted at room temperature for 1.5 hours and at 50°C for 5.5 hours. The reaction mixture was cooled to room temperature, diluted with saturated aqueous sodium chloride, and extracted with ethyl acetate. The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was purified by NH silica gel column chromatography (hexane:ethyl acetate = 1:1 to 1:2) to give the titled compound (0.14 g) as a light-green oil.

<sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>) δ:  
1.66-1.78 (m, 2H), 3.25 (s, 3H), 3.26-3.39 (m, 4H), 3.40-3.51 (m, 4H), 7.00-7.10 (m, 4H), 7.40 (d, *J* = 10.7 Hz, 1H), 8.42 (d, *J* = 10.7 Hz, 1H), 9.76 (s, 1H)

## Example 6

Preparation of N-{4-[6-(N,N-dimethylamino)hexyl]phenyl}-N'-hydroxyformamidine

Starting with 5-(N,N-dimethylamino)pentanol instead of diethylene glycol monomethyl ether, the same procedure as used in Example 5 was repeated to synthesize the titled compound.

Melting point: 96.0-97.0°C (colorless powder)

Example 7

Preparation of N-{4-[2-(5,5-dimethyl-[1,3]dioxan-2-yl)ethyl]phenyl}-N'-hydroxyformamidine

(1) To a solution of ethyl 4-nitrocinnamate (3.18 g,  
5 14.4 mmol) in tetrahydrofuran (30 ml), diisobutylaluminum  
hydride (1.0 M in toluene; 14.4 ml, 14.4 mmol) was added  
dropwise at -78°C and stirred at -78°C for 1 hour. After  
further addition of diisobutylaluminum hydride (1.0 M in  
toluene; 28.7 ml, 28.7 mmol), the reaction mixture was  
10 reacted at -78°C for 30 minutes. Aqueous hydrochloric acid  
(1M) was gradually added to the reaction mixture, followed  
by extraction with ethyl acetate. The organic layer was  
dried over anhydrous magnesium sulfate and concentrated  
under reduced pressure. The resulting residue was purified  
15 by silica gel column chromatography (hexane:ethyl acetate =  
2:1 to 1:3) to give 3-(4-nitrophenyl)propyl-2-en-1-ol (2.16  
g) as a yellow powder.

Melting point: 124.5-126.0°C

(2) To a solution of 3-(4-nitrophenyl)propyl-2-en-1-ol  
20 (0.67 g, 3.8 mmol) in methylene chloride (40 ml), activated  
manganese dioxide (5.01 g) was added and reacted at room  
temperature for 2 hours. The insoluble materials were  
filtered on celite and washed with chloroform. The  
filtrate was concentrated under reduced pressure and the  
25 resulting residue was purified by silica gel column  
chromatography (hexane:ethyl acetate = 6:1 to 3:1) to give  
3-(4-nitrophenyl)propenal (0.55 g) as a light-yellow powder.  
Melting point: 140.0-141.5°C



(3) To a solution of 3-(4-nitrophenyl)propenal (0.51 g, 2.9 mmol) in benzene (40 ml), p-toluenesulfonic acid monohydrate (0.059 g, 0.3 mmol) and 2,2-dimethylpropane-1,3-diol (0.36 g, 3.5 mmol) were added and reacted under heating at reflux for 5 hours. The reaction mixture was cooled to room temperature, followed by the addition of saturated aqueous sodium bicarbonate (100 ml), and then, extracted twice with ethyl acetate (100 ml). The combined organic layers were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane:ethyl acetate = 9:1 to 6:1) to give 5,5-dimethyl-2-[2-(4-nitrophenyl)vinyl]-[1,3]dioxane (0.73 g) as a colorless powder.

Melting point: 86.5-88.5°C

(4) A solution of 5,5-dimethyl-2-[2-(4-nitrophenyl)vinyl]-[1,3]dioxane (0.64 g, 2.4 mmol) in methanol (20 ml) was stirred in the presence of 5% palladium-activated charcoal (0.26 g) under a hydrogen atmosphere at room temperature for 4 hours. After confirming the disappearance of starting materials by TLC analysis, the insoluble materials were removed by filtration using celite and the filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane:ethyl acetate = 4:1 to 2:1) to give 4-[2-(5,5-dimethyl-[1,3]dioxan-2-yl)ethyl]phenylamine (0.48 g) as a colorless powder. Melting point: 93.5 to 94.5°C

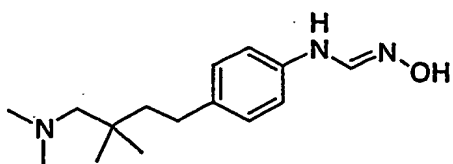
(5) To a solution of 4-[2-(5,5-dimethyl-[1,3]dioxan-2-yl)ethyl]phenylamine (0.32 g, 1.4 mmol) in methanol (10 ml), N,N-dimethylformamide dimethyl acetal (0.33 g, 2.8 mmol) was added and reacted at 100°C for 4 hours. After cooling to room temperature, the reaction mixture was mixed with hydroxylamine monohydrochloride (0.14 g, 1.9 mmol) and stirred overnight at room temperature. After addition of hydroxylammonium chloride (0.19 g, 2.8 mmol), the reaction mixture was reacted at room temperature for 1 hour and at 60°C for 6.5 hours. The reaction mixture was cooled to room temperature, followed by the addition of saturated aqueous sodium chloride, and then, extracted with ethyl acetate. The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was purified by NH silica gel column chromatography (hexane:ethyl acetate = 2:1 to 1:2) to give the titled compound (0.25 g) as a colorless powder. Melting point: 159.0-160.0°C

#### 20 Example 8

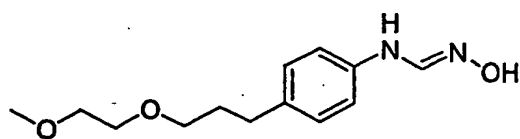
Preparation of N-{4-[3-(5,5-dimethyl-[1,3]dioxan-2-yl)propyl]phenyl}-N'-hydroxyformamidine

Starting with 4-(4-nitrophenyl)butyric acid instead of ethyl 4-nitrocinnamate, the same procedure as used in Example 7 was repeated to synthesize the titled compound. Melting point: 138.0-140.0°C (colorless powder)

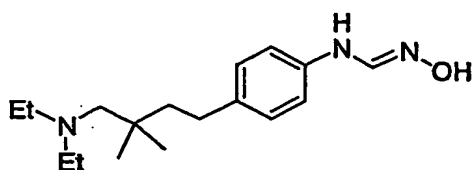
The structural formulae of the compounds synthesized in Examples 1 to 8 will be shown below.



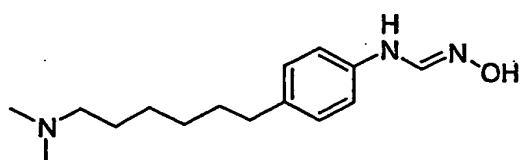
Example 1



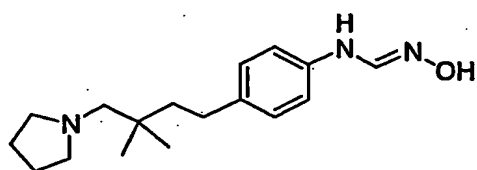
Example 5



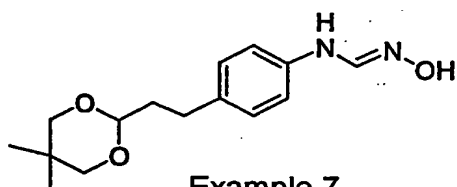
Example 2



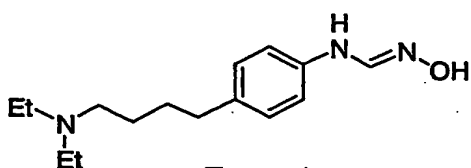
Example 6



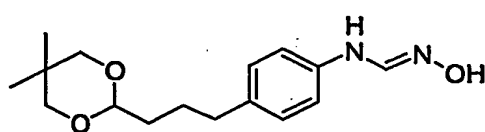
Example 3



Example 7



Example 4



Example 8

Test Example [Inhibitory effect against human kidney  
microsome-derived 20-HETE-producing enzyme]

5        The compounds listed in the table above were tested  
for their inhibitory effect against 20-HETE production.

      This test was performed as described in J. Pharmacol.  
Exp. Ther., vol. 268, p. 474, 1994.

10       A test drug solution adjusted with DMSO to 1  $\mu$ M was  
added to 50 mM 3-morpholinopropanesulfonate (MOPS) buffer  
(pH 7.4) containing 5 mM magnesium chloride and 1 mM  
ethylenediaminetetraacetic acid disodium salt (EDTA). The

resulting mixture was further supplemented with a human kidney microsomal fraction (Human Cell Culture Center, Anatomic Gift Foundation) as an enzyme source, [5,6,8,9,11,12,14,15]tritium-arachidonic acid as a substrate, and NADPH as a coenzyme, followed by reaction at 37°C for 1.5 hours. After addition of formic acid to stop the reaction, the reaction mixture was supplemented with acetonitrile (final concentration: 50%). The level of 20-HETE production was measured by high performance liquid chromatography using an ODS column (Biosil C18, BioRad Laboratories) and a radioisotope detector. Assuming that the level of 20-HETE production in the absence of a test compound was set to 100%, the concentration required for the test compound to cause 50% inhibition of 20-HETE production was calculated (IC<sub>50</sub> value). Table 1 shows the result obtained.

Table 1

Compound	IC <sub>50</sub> (nM)
Compound of Example 1	10.5

#### INDUSTRIAL APPLICABILITY

The compounds according to the present invention have an inhibitory effect against 20-HETE production and are useful as therapeutic agents for 20-HETE-associated diseases in human subjects and animals, including various kidney diseases, cerebrovascular diseases and various cardiovascular diseases.